

Morphology and Phylogeny of Four Marine Scuticociliates (Protista, Ciliophora), with Descriptions of Two New Species: *Pleuronema elegans* spec. nov. and *Uronema orientalis* spec. nov.

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Abstract. The morphology and infraciliature of four marine scuticociliates, *Pleuronema elegans* spec. nov., *P. setigerum* Calkins, 1902, *P. grolierei* Wang *et al.*, 2008 and *Uronema orientalis* spec. nov., collected from China seas, were investigated through live observation and protargol staining methods. *Pleuronema elegans* spec. nov. can be recognized by the combination of the following characters: size *in vivo* 90–115 × 45–60 μm, slender oval in outline with a distinctly pointed posterior end; about 10 prolonged caudal cilia; consistently two preoral kineties and 18 or 19 somatic kineties; membranelle 2a double-rowed with its posterior end straight; membranelle 3 three-rowed; one macronucleus; marine habitat. *Uronema orientalis* spec. nov. is distinguished by the following features: *in vivo* about 40–55 × 20–30 μm with a truncated apical plate; consistently twenty somatic kineties; membranelle 1 single-rowed and divided into two parts which comprise four and three basal bodies respectively; contractile vacuole pore positioned at the end of the second somatic kinety; marine habitat. We also provide improved diagnoses for *P. grolierei* Wang *et al.*, 2008 and *P. setigerum* Calkins, 1902 based on current and previous reports. The small subunit rRNA gene of *U. orientalis*, *P. elegans*, *P. grolierei* and *P. puytoraci* were sequenced. Phylogenetic analyses indicate that *Uronema* and *Pleuronema* are not monophyletic.

Key words: Scuticociliates, *Pleuronema elegans* spec. nov., *Uronema orientalis* spec. nov., phylogeny.

INTRODUCTION

Investigations into scuticociliates have demonstrated that this assemblage is much more diverse than was

previously assumed (Thompson and Kaneshiro 1968; Foissner and Wilbert 1981; Foissner *et al.* 1994, 2013; Song and Wilbert 2002; Long *et al.* 2007; Song *et al.* 2007; Miao *et al.* 2008, 2009; Wilbert and Song 2008; Yi *et al.* 2009; Budiño *et al.* 2011; Fan *et al.* 2011a, b; Lobban *et al.* 2011; Pan *et al.* 2011, 2013d; Salinas *et al.* 2011; Seo *et al.* 2013; Whang *et al.* 2013). Many nominal species are insufficiently described and/or lack gene sequence data, and, consequently, further investi-

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gations of this group are needed using a combination of morphological and molecular data (Gao *et al.* 2010, 2012a, b, 2013, 2014).

Of the scuticociliates, *Pleuronema* comprises tens of nominal species, at least 20 of which have been studied using silver staining techniques (Grolière and Detcheva 1974; Small and Lynn 1985; Dragesco and Dragesco-Kernéis 1986; Agatha *et al.* 1993; Fernandez-Leborans and Novillo 1994; Wang *et al.* 2008a, b, 2009). Out of these, 18 have been described using silver staining techniques, notwithstanding this, some studies provide insufficient information to validate these species (Grolière and Detcheva 1974, Czapik and Jordan 1977, Foissner *et al.* 1994).

Uronema Dujardin, 1841, is another very common genus of scuticociliate, comprising many nominal species which are found worldwide in both freshwater and marine habitats (Kahl 1931; Czapik 1964; Thompson 1964, 1972; Perez-Uz and Song 1995; Song 2000). A number of new or little-known *Uronema* species have been isolated and reported since the end of the last century during faunistic surveys which have been conducted in the marine waters of China (Song *et al.* 2002, Pan *et al.* 2010).

Overall, many scuticociliates have been identified and described during studies of the ciliate fauna in both the north and south China seas (Song and Wilbert 2000, Song *et al.* 2002, Ma and Song 2003; Fan *et al.* 2011a, b; Pan *et al.* 2013d). As a new contribution, this study presents the morphology and phylogeny of four scuticociliate species.

MATERIALS AND METHODS

Ciliate collection and identification: *Pleuronema elegans* spec. nov. was collected on 15 May 2012 from No. 1 swimming beach, Qingdao, northern China (36°06'N; 120°32'E), when the water temperature was about 19°C, pH 7.6 and salinity 31‰. A 10 cm-deep hole was dug in the sand into which seawater gradually seeped. The sample comprised a mixture of seawater and sand from the bottom of the hole (Fig. 1A).

Uronema orientalis spec. nov. was collected on 13 April 2012 from the beach near Sculpture Garden (36°4'N; 120°29'E), Qingdao, when the water temperature was about 15°C, pH 7.8 and salinity 29‰. The method of collection was the same as that for *Pleuronema elegans* spec. nov. (Fig. 1B).

Pleuronema setigerum Calkins, 1902 was collected from a mangrove wetland in Shenzhen, Guangdong Province (22°30'N; 114°37'E) on 1 December 2010 when the water temperature was 21°C, salinity 19‰, and pH 8.0 (Fig. 1D). *Pleuronema grolierei* Wang *et al.*, 2008 was collected from No. 1 swimming beach at

the site that is further into the sea than that of *P. elegans*, Qingdao (36°06'N; 120°32'E) on 6 May 2010 when the water temperature was 14°C, salinity 30‰, and pH 7.4. In both of these latter cases, sand (top 5 cm layer), or sediment, plus seawater were taken from the site (Fig. 1C).

Individuals were observed *in vivo* using differential interference contrast microscopy. Protargol staining was used in order to reveal the infraciliature (Pan *et al.* 2013a, b, c). Counts and measurements of stained specimens were performed at magnifications of 100–1250×. Drawings were carried out with the help of a camera lucida. Systematics and terminology are mainly according to Lynn (2008).

DNA extraction, PCR amplification and sequencing: Genomic DNA of the species *Uronema orientalis* spec. nov., *Pleuronema elegans* spec. nov., *P. grolierei*, and *P. puytoraci* were extracted from cells using the DNeasy Tissue kit (Qiagen, CA). The species *P. puytoraci* is from the Hong Kong population described in Pan *et al.* (2011). We failed to extract DNA from *P. setigerum* due to the low number of specimens of this species. The PCR amplifications of SSU-rDNA were performed with the universal primers (Medlin *et al.*, 1988). Purified PCR product of the appropriate size was inserted into the pMD™18-T vector (Takara Biotechnology, Dalian Co., Ltd.) and sequenced on an ABI-PRISM 3730 automatic sequencer (Applied Biosystems).

Sequence availability and phylogenetic analyses: With the exception of the four newly characterized SSU rRNA gene sequences (*Pleuronema elegans* spec. nov., *P. grolierei* Wang *et al.*, 2008, *P. puytoraci* Grolière & Detcheva, 1974 and *Uronema orientalis* spec. nov.), the rest of the sequences used in the study were obtained from the GenBank database. Sequences were aligned using Clustal W implemented in BioEdit 7.0 (Hall 1999). Bayesian inference (BI) analyses were performed with MrBayes v.3.1.2 (Ronquist and Huelsenbeck 2003) using the GTR + I + G model selected by MrModeltest v.2.2 (Nylander 2004) according to the AIC criterion. Markov Chain Monte Carlo (MCMC) simulations were run with two sets of four chains for 2,500,000 generations, with trees sampled every 100 generations. The first 25% of sampled trees were discarded as burn in. All remaining trees were used to calculate posterior probabilities using a majority rule consensus. Maximum likelihood (ML) trees were constructed with PhyML v.2.4.4 (Guindon and Gascuel 2003) using the best model selected by Modeltest v.3.4 (Posada and Crandall 1998). The reliability of internal branches was assessed using nonparametric bootstrapping with 1000 replicates. Phylogenetic trees were visualized with Tree-View v.1.6.6 and MEGA v.4 (Tamura *et al.* 2007).

RESULTS AND DISCUSSION

Subclass Scuticociliatia Small, 1967

Family Pleuronematidae Kent, 1881

Genus *Pleuronema* Dujardin, 1836

Pleuronema elegans spec. nov. (Fig. 2, Table 1)

Diagnosis: Size *in vivo* 90–115 × 45–60 μm with a distinctly pointed posterior end; contractile vacuole located dorsally near posterior end; about 10 prolonged

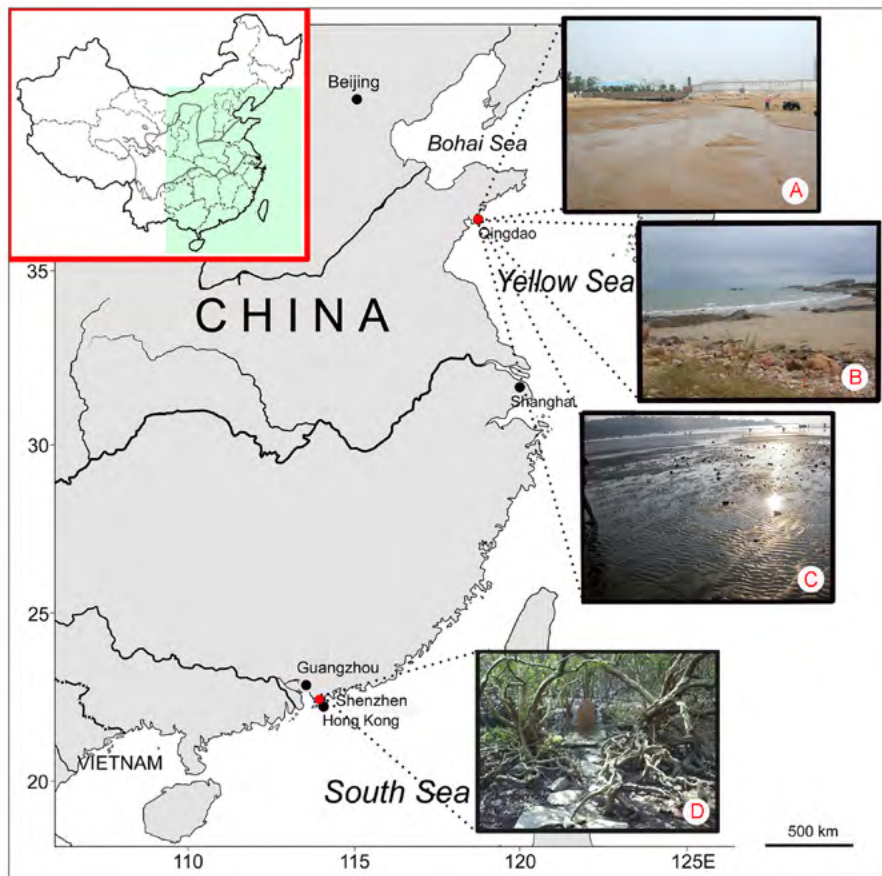


Fig. 1. Map and photographs of biotopes (A–D) in which the samples were collected. **A** – no. 1 swimming beach, Qingdao (36°06'N; 120°32'E); **B** – beach near Sculpture Garden, Qingdao (36°4'N; 120°29'E); **C** – coastal area of no. 1 swimming beach, Qingdao (36°06'N; 120°32'E), the site that is further into the sea than that of (A); **D** – a mangrove wetland in Shenzhen, Guangdong Province (22°30'N; 114°37'E).

caudal cilia; consistently two preoral and 18 or 19 somatic kineties; membranelle 1 with a length about 50% that of the anterior part of membranelle 2 which is double-rowed with its posterior end straight but not hook-shaped; marine habitat.

Type locality: Swimming beach, Qingdao, northern China (36°06'N; 120°32'E).

Type slides: The holotype slide (registration number: PXM-20120515) and one paratype slide (registration number: NHMUK 2013.8.15.1) with protargol stained-specimens are deposited in the Laboratory of Protozoology, Ocean University of China (OUC) and the Natural History Museum, London, respectively.

Etymology: This new form named '*elegans*' refers to its elegant body shape.

Description: The body is about $100 \times 50 \mu\text{m}$ *in vivo*, slender oval in outline, with a distinctly pointed posterior end (Figs 2A, H, I). Buccal field cavity is about 70% of body length with a conspicuous, sail-like, undulating membrane (Fig. 2I). Pellicle is rigid and slightly notched with closely arranged extrusomes, which is about $3 \mu\text{m}$ long (Fig. 2B). Cytoplasm is colourless to slightly grayish, packed with large amounts of green ingested algae and shining globules of varying size, food vacuoles which are usually large and filled with bacteria, and blue irregularly-shaped crystals ($< 6 \mu\text{m}$ in diameter) (Figs 2A, H, I). One spherical macronucleus, about $32 \times 32 \mu\text{m}$, located in anterior half of cell. No micronucleus is observed (Fig. 2P). Single contractile vacuole is about $10 \mu\text{m}$ in diameter, located slightly dorsally near posterior end of cell (Fig. 2A).

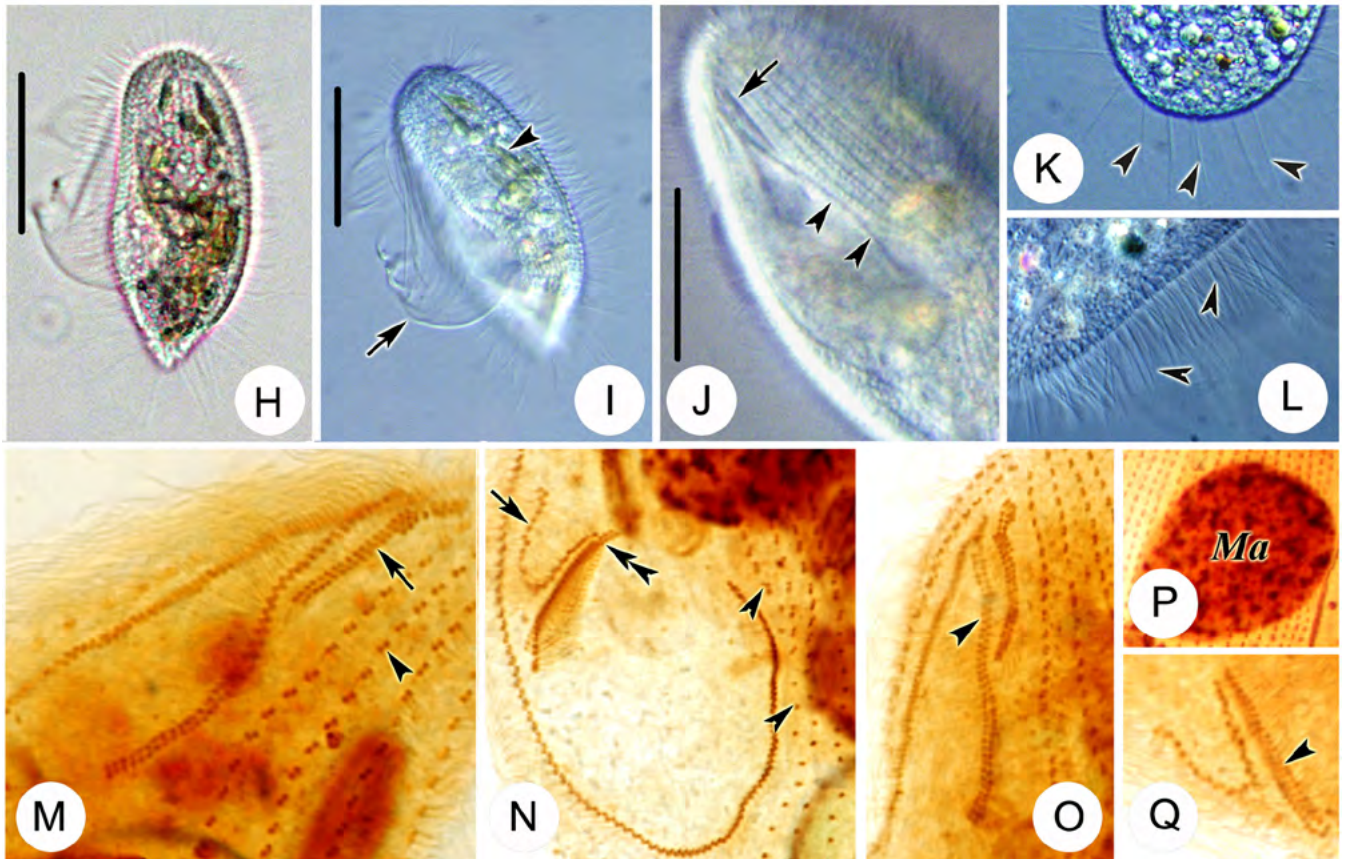
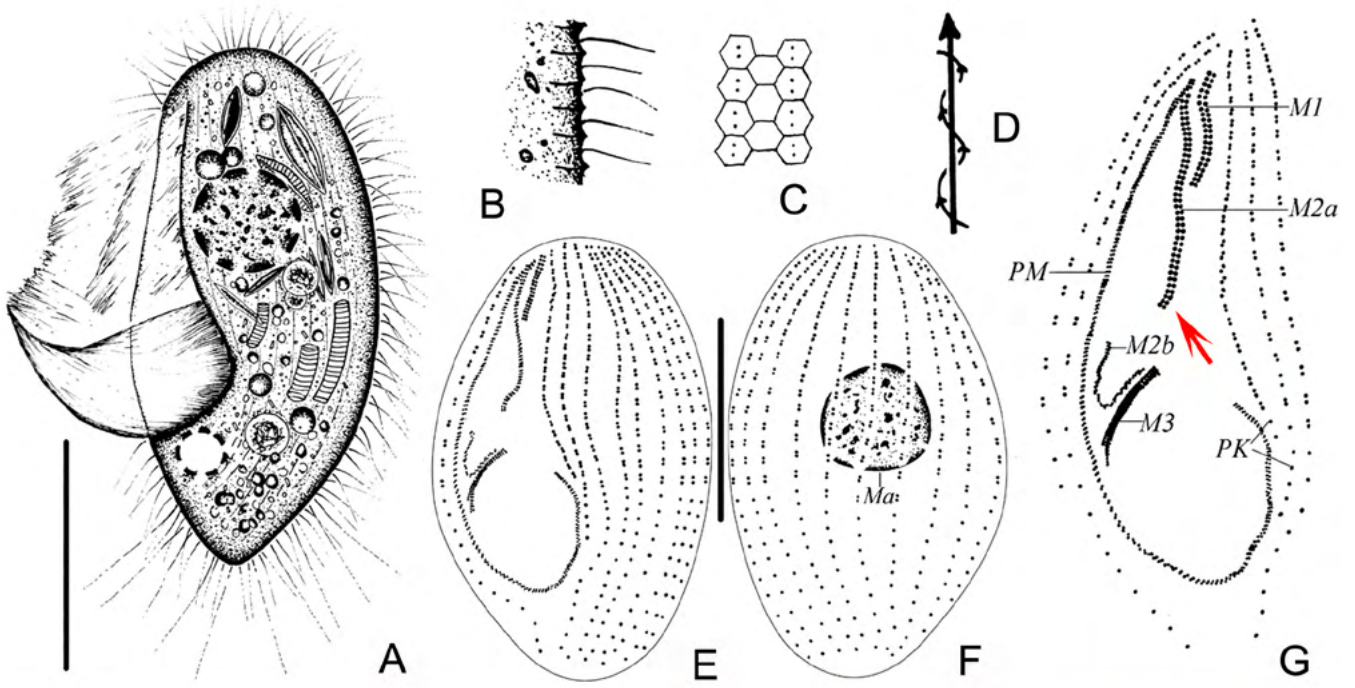


Table 1. Morphometric characterization of *Pleuronema elegans* spec. nov. (upper row), *P. setigerum* Calkins, 1902 (second row), *P. grolierei* Wang *et al.*, 2008 (third row) and *Uronema orientalis* spec. nov. (lower row). Data based on protargol stained specimens. All measurements in μm . Abbreviations: CV – coefficient of variation in %; Max – maximum; Mean – arithmetic mean; Min – minimum; n – number of individuals examined; SD – standard deviation.

Character	Min	Max	Mean	SD	CV	n
Body length	104	122	111.7	4.8	4.3	21
	42	58	50.2	5.1	10.2	19
	32	45	38.4	7.1	3.7	21
	74	82	80.3	5.3	6.2	21
Body width	58	66	62.4	2.7	4.3	21
	26	35	30.4	2.9	9.5	19
	18	24	21.1	4.3	4.6	21
	28	39	34.2	6.1	5.6	21
Number of somatic kineties	18	19	18.2	0.4	2.2	20
	20	20	20.0	0	0	18
	12	14	12.8	3.2	2.7	18
	18	22	20.2	6.2	2.6	19
Number of preoral kineties	2	2	2.0	0	0	15
	–	–	–	–	–	–
	5	6	5.2	3.6	2.7	16
	1	1	1.0	0	0	12
Number of kinety rows in M3	3	3	0	0	0	15
	3	3	0	0	0	14
	3	3	0	0	0	14
	2	2	0	0	0	13
Length of macronucleus	29	36	32.4	2	6.2	21
	16	19	16.5	0.9	5.5	19
	9	12	11.2	0.8	4.9	21
	14	17	14.8	7.1	6.2	21
Width of macronucleus	28	32	31	1.6	5.2	21
	14	16	14.7	0.7	4.8	19
	10	13	8.5	3.1	5.7	21
	14	18	15.9	2.7	4.8	21

◀◀

Fig. 2. *Pleuronema elegans* spec. nov. *in vivo* (A, B, D, H–L), after protargol (E–G, M–Q) and silver nitrate staining (C). **A, H** – ventral view of a representative individual; **B** – detailed view of cortex to demonstrate arrangement of extrusomes; **C** – part of silverline system; **D** – swimming trace; **E, F** – ventral (E) and dorsal (F) views of the same specimen, showing infraciliature and nuclear apparatus; **G** – detailed structure of the buccal area, arrow shows the straight posterior end of M2a; **I** – ventral view, arrow marks paroral membrane and arrowhead shows ingested algae; **J** – ventral view, arrow shows membranelle 1, arrowheads mark the anterior part of membranelle 2; **K** – ventral view, arrowheads point to caudal cilia; **L** – ventral view, arrowheads mark somatic cilia; **M** – infraciliature of ventral side, arrow indicates membranelle 1, arrowhead refers to paired basal bodies; **N** – posterior region, arrow shows V-shaped posterior part of membranelle 2, arrowheads point to preoral kineties, double-arrowhead indicates membranelle 3; **O** – anterior region, arrowhead shows the anterior part of membranelle 2; **P** – macronucleus; **Q** – detailed view of membranelle 3 (arrowhead). M1, 3 – membranelles 1 and 3; M2a – the anterior part of membranelle 2; M2b – the posterior part of membranelle 2; Ma – macronucleus; PK – preoral kinety; PM – paroral membrane. Scale bars: A, E, F, H, I = 50 μm , J = 20 μm .

Somatic cilia is about 12 μm long (Figs 2A, L). There are about ten prolonged caudal cilia, each is about 30 μm in length (Fig. 2K).

The cell swims moderately fast while rotating about main body axis, sometimes lying motionless along substrate such as bottom of Petri dish or detritus (Fig. 2D).

There are eighteen or 19 somatic kineties, which are composed of dikinetids in anterior 60% of body and monokinetids in posterior third, extending almost the entire length of the cell, terminating anteriorly at a small glabrous apical plate (Figs 2E, F). There are consistently two preoral kineties to the left of the buccal field (Figs 2G, N).

Oral apparatus is typical for genus: M1 comprises two longitudinal rows of basal bodies, the length of which is about 50% that of the anterior part of M2a (Figs 2G, M, O). M2a is double-rowed with its posterior end straight; posterior part of M2b is V-shaped, and is distinctly separated from M2a (Figs 2G, N). M3 is three-rowed with a similar length to that of M1 (Figs 2G, Q). Length of paroral membrane is about 70% of body length. Silverline system is typical for the genus with a near-hexagonal honeycomb pattern (Fig. 2C).

SSU rRNA gene sequence: The SSU rRNA gene sequence of *Pleuronema elegans* spec. nov. has been deposited in the GenBank database with the accession number, length and G+C content as follows: KF840518, 1661 bp, 42.75%.

Remarks and comparison: Based on its conspicuously pointed posterior end and marine habitat, *Pleuronema elegans* spec. nov. most resembles two nominal species: *P. czapikae* Wang *et al.*, 2008 and *P. tardum* Czapik & Jordan, 1977 and can be readily separated from other congeners.

Pleuronema elegans spec. nov. can be clearly distinguished from *P. czapikae* Wang *et al.*, 2008 through its different body shape (slender oval in outline, with a distinctly pointed posterior end in *P. elegans* vs. elongate-elliptical in outline, almost parallel-sided with both ends slightly pointed in *P. czapikae*), fewer somatic kineties (18–19 vs. 29–35 in *P. czapikae*) and M2a double-rowed with its posterior end straight (vs. mostly two-rowed but with a short section that is single-rowed, posterior end invariably hook-shaped in *P. czapikae*) (Wang *et al.* 2008b).

Pleuronema elegans spec. nov. differs from *P. tardum* in having more preoral kineties (two vs. one in *P. tardum*), less somatic kineties (18–19 vs. 40–50) and a different ratio of M1 and M3 to the anterior part of

M2a (50% vs. M1 and M3 very short and M2a extremely long in *P. tardum*) (Czapik and Jordan 1977).

***Pleuronema setigerum* Calkins, 1902 (Figs 3 I–M; Table 1)**

Since first reported, this species has been re-described on four occasions (Kahl 1931, Noland 1937, Borror 1963, Pan *et al.* 2010). Some new characters were found in the Shenzhen population and hence an improved diagnosis of this species is supplied here based on both previous and present studies.

Improved diagnosis: *In vivo* 25–50 \times 10–30 μm in size, slender oval in outline; buccal field occupying four-fifths of body length; about 9–13 prolonged caudal cilia; three to five preoral kineties and 12–22 somatic kineties; M1 about 20% of the anterior part of M2a in length, consisting of three longitudinal rows of basal bodies; posterior end of M2a ring-like; contractile vacuole subcaudally positioned; one macronucleus; marine habitat.

Description of the Shenzhen population: *In vivo* 25–40 \times 10–20 μm , slender oval in outline, widest at mid-body (Fig. 3I). Ventral side almost flat, dorsal side convex. Buccal field cavity about four-fifths of body length (Fig. 3I). Extrusomes 3 μm long, lying beneath notched pellicle and closely arranged between ciliary rows (Fig. 3J). Cytoplasm colourless to slightly grayish, containing many shining globules of varying size (3–5 μm across), food vacuoles (4–6 μm across) and irregularly-shaped crystals (mostly 3–4 μm across) (Fig. 3I, J). One spherical macronucleus, about 16 \times 15 μm , located in anterior 1/3 of cell, usually with many globular nucleoli. Single contractile vacuole about 10 μm in diameter, located subcaudally near dorsal cell margin (Fig. 3K). Somatic cilia about 8 μm long (Fig. 3I). About thirteen prolonged caudal cilia, each about 20 μm in length (Fig. 3K).

Swims moderately fast while rotating about main body axis, sometimes drifting or lying motionless on debris for short periods (Fig. 3M).

Twelve to 14 somatic kineties, composed of paired basal bodies in anterior three-quarters of the body and monokinetids in the posterior quarter, extending almost the entire length of the cell (Fig. 3M). Five or six preoral kineties to left of buccal field.

Oral apparatus typical for genus: M1 with one short and two longer rows of basal bodies; M2a mostly two-rowed but with a middle section that is single-rowed in a ‘zigzag’ pattern, with its posterior end characteristically ring-like (Fig. 3L). M2b V-shaped, distinctly

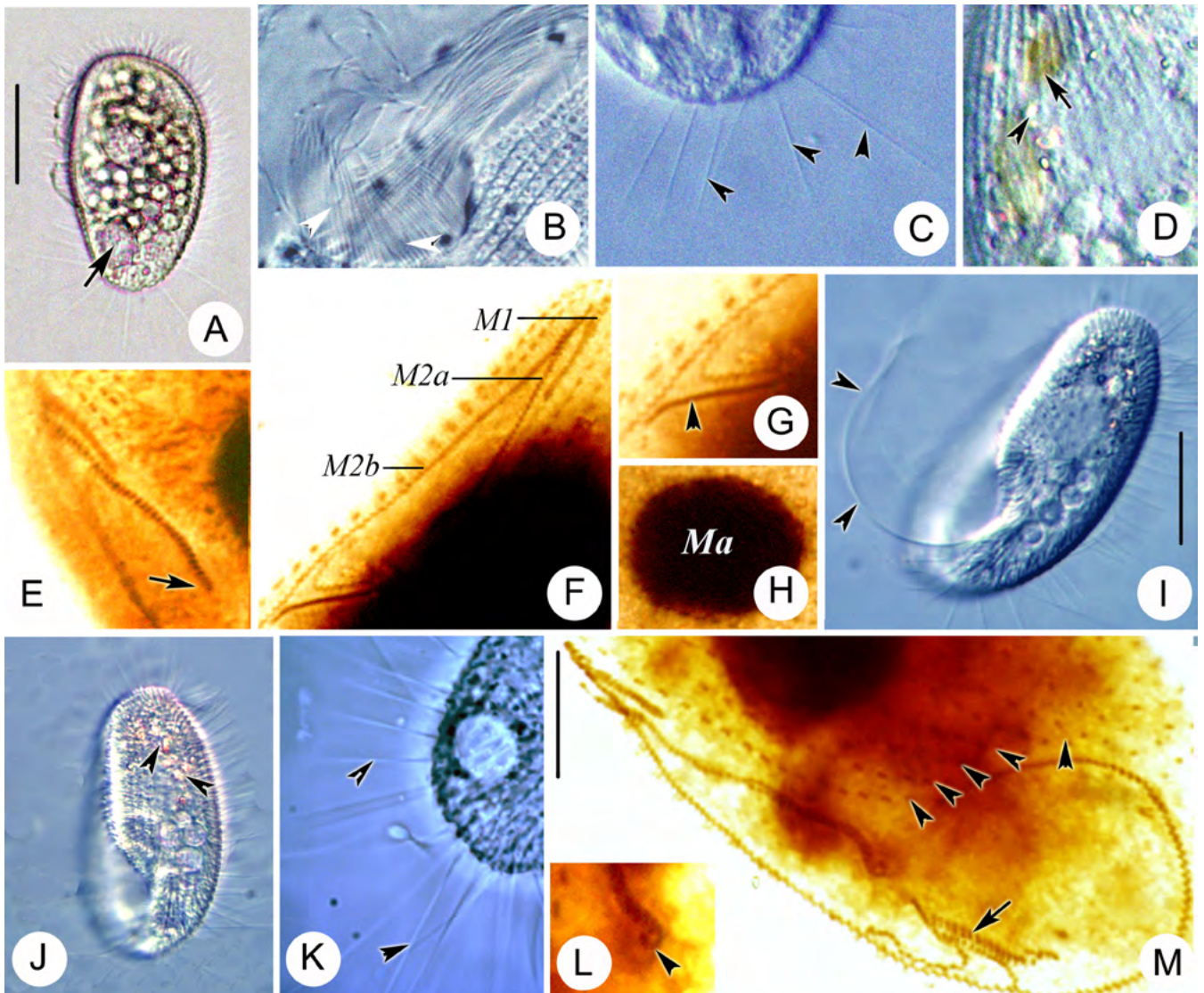


Fig. 3. *Pleuronema grolierei* Wang *et al.*, 2008 (A–H) and *P. setigerum* Calkins, 1902 (I–M) *in vivo* (A–D, I–K) and after protargol (E–H, L, M). **A, I** – ventral views of typical individuals, arrow in (A) shows contractile vacuole, arrowheads in (I) mark paroral membrane; **B** – ventral view, arrowheads mark oral cilia; **C, K** – ventral views, arrowheads point to caudal cilia; **D** – ventral view, arrow marks ingested algae, arrowhead shows irregularly-shaped crystals; **E** – anterior region, arrow shows the anterior part of membranelle 2; **F** – detailed structure of the buccal area; **G** – posterior region, arrowhead points to membranelle 3; **H** – macronucleus; **J** – ventral view, arrowheads show irregularly-shaped crystals; **L** – posterior region, arrowhead indicates the ring-like posterior end of M2a; **M** – detailed structure of the buccal area, arrow marks membranelle 3, arrowheads show preoral kineties. M1 – membranelle 1; M2a – the anterior part of membranelle 2; M2b – the posterior part of membranelle 2; Ma – macronucleus. Scale bars: A, I = 20 μm , M = 5 μm .

separated from M2a; M3 three-rowed (Fig. 3M). Paroral membrane about 80% of cell length.

Comparison and remarks: *Pleuronema setigerum* was first reported by Calkins in 1902 and then redescribed by Kahl (1931) who cited Calkins's drawing. Borror (1963) described its infraciliature but gave only a diagram of the buccal morphology, then Small (1964)

provided a detailed line drawing based on Borror's protargol stained specimen. Pan *et al.* (2010) redescribed this form and provided an improved diagnosis based both on the Qingdao population and the previous studies. The Shenzhen population is very similar to the previous ones in both living and infraciliature data, except for having a smaller body size (25–40 \times 10–20 μm vs.

30–50 × 15–30 µm), fewer somatic kineties (12–14 vs. 14–22) and more preoral kineties (five or six vs. three or five). We consider these variations to be population-dependent, and thus treat the Shenzhen population as conspecific with other populations of *P. setigerum*.

***Pleuronema grolierei* Wang *et al.*, 2008 (Figs 3A–H; Table 1)**

Pleuronema grolierei was originally described by Wang *et al.* (2008b), but the molecular information of this form was absent in his study. Based on the current study, an improved diagnosis of this species is provided, with new characters and SSU rRNA gene sequence data.

Improved diagnosis: Size *in vivo* 60–80 × 20–40 µm with an oval to elliptical body shape; buccal field occupying two-thirds of body length; single contractile vacuole located slightly ventrally in the posterior fourth; ten to fifteen prolonged caudal cilia in posterior half of body; cilia in PM uniquely short and inconspicuous; one preoral and 18 to 32 somatic kineties; all membranelles consisting of two rows of basal bodies; the posterior end of the anterior fragment of M2a straight; M2a about twice as long as M3; PM about two-thirds of cell length; single spherical macronucleus and marine habitat.

Description of the Qingdao population: *In vivo* about 60–70 × 25–35 µm, elongate oval to elliptical in outline, both ends broadly rounded (Fig. 3A). Buccal field large with shallow buccal cavity, occupying about two-thirds of body length and almost one-third of body width (Fig. 3A). Cilia in both PM and membranelles obviously short (about 20 µm long) (Fig. 3B). Pellicle rigid and slightly notched with extrusomes about 3 µm long, closely arranged beneath. Cytoplasm colourless to slightly grayish, packed with many shining globules of varying size, food vacuoles which are usually large and filled with indefinable contents (Fig. 3D). Single spherical macronucleus, about 15 µm in diameter (Fig. 3H). Contractile vacuole about 20 µm in diameter, located slightly ventrally in posterior quarter of the cell (Fig. 3A). Somatic cilia usually about 10 µm long; about ten caudal cilia, approximately 20 µm long (Fig. 3C). Movement moderately fast, rotating about main body axis, somewhat drifting and wobbling and then motionless for short periods.

Eighteen to 22 somatic kineties, extending over the entire length of the cell and terminating at the apical end around a large glabrous apical plate. All kineties composed of dikinetids in anterior half of the body and monokinetids in posterior half. One preoral kinety to the left of the buccal field. Oral apparatus as shown in

Figs 3E–G; the anterior M1 about the same length as M3; M2a double-rowed with its posterior end straight (Figs 3E, G). All the membranelles composed of two longitudinal rows of basal bodies. PM about two-thirds of cell length with its posterior end strongly curved around the posterior margin of buccal area.

SSU rRNA gene sequence: The SSU rRNA gene sequence of *Pleuronema grolierei* Wang *et al.*, 2008 has been deposited in the GenBank database with the accession number, length and G+C content as follows: KF840519, 1738 bp, 43.44%.

Comparison and remarks: Our population is very similar to previous populations (Wang *et al.* 2008a) in both living and infraciliature data, except for minor differences in the number of somatic kineties (18–22 in the present study vs. 24–32) and the number of caudal cilia (ten in the present population vs. 15). Consequently, these two forms are conspecific.

Family Uronematidae Thompson, 1964

Genus *Uronema* Dujardin, 1841

***Uronema orientalis* spec. nov. (Fig. 4; Table 1)**

Diagnosis: *In vivo* about 40–55 × 20–30 µm with a truncated apical plate; buccal field about 50% of body length; consistently twenty somatic kineties; membranelle 1 (M1) one-rowed, divided into two parts: the anterior part (M1a) and the posterior part (M1b), comprising four and three basal bodies, respectively; contractile vacuole caudally positioned near ventral margin; contractile vacuole pore (CVP) positioned at end of the second somatic kinety; marine habitat.

Type locality: A beach near Sculpture Garden (36°4'N; 120°29'E), Qingdao, China.

Type slides: The holotype slide (registration number: PXM-2012041301) and one paratype slide (registration number: NHMUK 2013.8.15.2) with protargol stained-specimens are deposited in the Laboratory of Protozoology, OUC and the Natural History Museum, London, respectively.

Dedication and etymology: The species receives its name '*orientalis*' from the locality where it was isolated.

Description: Size *in vivo* about 40–55 × 20–30 µm, elongate-elliptical in outline (Figs 4A, E). Anterior end flat, with an apical plate, dorsal posterior area broadly rounded (Figs 4A, E). Buccal field about 50% of body length (Fig. 4A). Pellicle smooth, without ridges (Figs 4E–G). Extrusomes bar-shaped, about 4 µm long, and sparsely arranged beneath pellicle. Cytoplasm colourless to grayish, containing several to

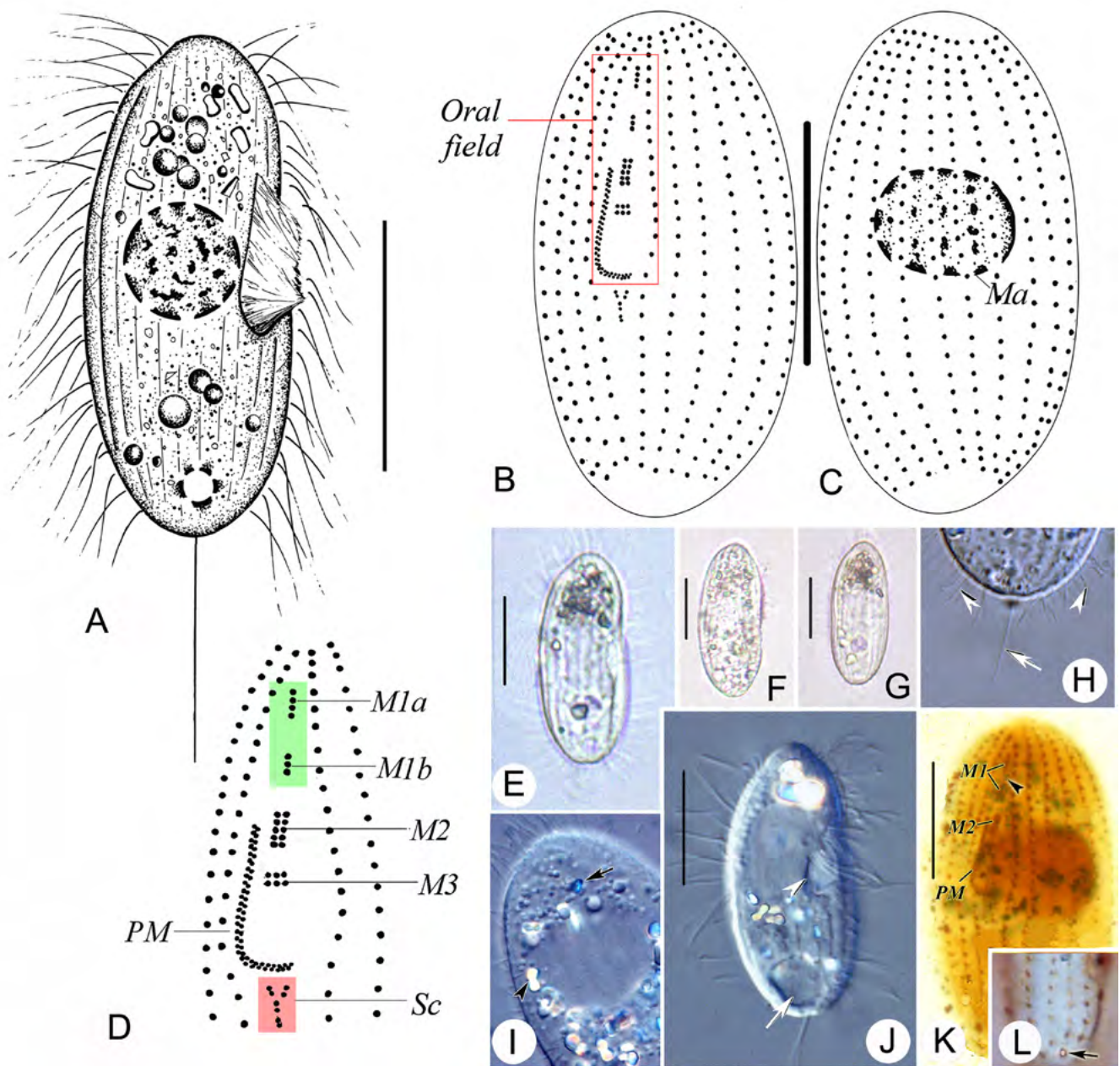


Fig. 4. *Uronema orientalis* spec. nov. *in vivo* (A, E–J), after protargol (B–D, K) and silver nitrate staining (L). A, E – ventral views of a typical cell; B, C – ventral (B) and dorsal (C) views of the same specimen, showing infraciliature and nuclear apparatus; D, K – detailed infraciliature of buccal area, arrowhead in (K) shows the gap between the anterior part and posterior parts of membranelle 1; F, G – ventral views, to show different body shapes; H – posterior region of cell, arrow points to caudal cilia, arrowheads show somatic cilia; I – ventral view, arrow refers to blue irregularly-shaped crystal, arrowhead indicates dumbbell-shaped crystal; J – ventral view, arrow shows contractile vacuole, arrowhead marks buccal field; L – ventral view, arrow shows contractile vacuole pore. M1, 2, 3 – membranelles 1, 2 and 3; M1a – the anterior part of membranelle 1; M1b – the posterior part of membranelle 1; Ma – macronucleus; PM – paroral membrane; Sc – scutica. Scale bars: A, B, C = 50 μ m; E–G, J, K = 80 μ m.

many large (ca 5 μm across) food vacuoles and dumb-bell-shaped crystals, which are usually 4 μm long (Figs 4A, I, J). Single macronucleus oval to spherical, centrally located (Fig. 4C). Contractile vacuole moderately large, 5 μm in diameter, caudally positioned (Figs 4A, J). Somatic cilia about 10 μm long, densely arranged; single caudal cilium approximately 20 μm long (Figs 4G, H). Swimming moderately fast while rotating about main body axis, sometimes crawling on debris, or resting on the bottom.

Consistently twenty somatic kineties arranged longitudinally, which usually have monokinetids in the entire length of each row (Figs 4B, C, K). Buccal apparatus as shown in Figs 4D, K: M1 one-rowed, divided into two parts: the anterior part (M1a) and the posterior part (M1b) comprising four and three basal bodies, respectively. M2 composed of two longitudinal rows of basal bodies; M3 comprising three longitudinal rows (Figs 4D, K). Paroral membrane on right of buccal cavity terminating halfway along M2 (Figs 4D, K). Scutica consisting of four pairs of basal bodies (Figs 4D, K). Contractile vacuole pore positioned at the end of second somatic kinety (Fig. 4L).

SSU rRNA gene sequence: The SSU rRNA gene sequence of *Uronema orientalis* spec. nov. has been deposited in the GenBank database with the accession number, length and G+C content as follows: KF840517, 1657 bp, 42.37%.

Remarks and comparison: Considering the morphology, infraciliature and habitat, three species have similarities with our new species: *Uronema marinum* Dujardin, 1841, *U. elegans* Maupas, 1883 and *U. heteromarinum* Pan *et al.*, 2010.

Though *Uronema marinum* is similar to *U. orientalis* in body shape and the conspicuous extrusomes, it can be distinguished by the patterns of M1 (one row with 3–6 basal bodies in *U. marinum* vs. divided into two parts and comprising four and three basal bodies, respectively in *U. orientalis*), the number of somatic kineties (12–14 vs. 20 in *U. orientalis*), and the location of the contractile vacuole pore (at posterior end of kinety 2 in *U. marinum* vs. at posterior end of kinety 1 in *U. orientalis*) (Pan *et al.* 2010).

Compared with *Uronema orientalis*, *U. elegans* is distinguished by the ratio of body length to width (1.5: 1 vs. 2.5: 1 in *U. orientalis*) and more somatic kineties (23–26 vs. 20) (Song *et al.* 2002).

Uronema heteromarinum differs from *U. orientalis* in having reticulate ridges on a notched pellicle and

fewer somatic kineties (15–16 vs. 20 in *U. orientalis*) (Pan *et al.* 2010).

Phylogenetic analyses (Fig. 5)

The topologies of the SSU rRNA gene trees constructed using Bayesian inference and maximum-likelihood analyses are similar and therefore a single topology is presented here with support values from both algorithms (Fig. 5). Our phylogenetic trees show that all the *Pleuronema* species included in the analyses fall into two clades, which are separated by the genus *Schizocalyptra*. *Pleuronema elegans* spec. nov., *P. grolierei*, and *P. puytoraci* are unambiguously placed in the “core” *Pleuronema* clade. The new form *P. elegans* spec. nov. clusters with *P. coronatum* (JX310014) with full support while *P. puytoraci* groups with *P. setigerum* (FJ848874) (1.00 BI, 99 % ML). *Pleuronema grolierei* shows a close relationship with *P. setigerum* (JX310015) and *P. cf. setigerum* (FJ848875). With three more sequences added, *Pleuronema* is still not monophyletic, being interrupted by the genus *Schizocalyptra*, which concurs with previous studies (Gao *et al.* 2013). However, the support values for the branching of pattern of *Pleuronema* and *Schizocalyptra* are very low and the hypothesis that all *Pleuronema* spp. cluster together is not rejected by the AU test in Gao *et al.* (2013). As the phylogenetic trees show, *Uronema orientalis* spec. nov. clusters in the clade containing the type species *U. marinum* with moderate support (0.81 BI, 89 % ML), which reinforces the assignment of this species in the genus *Uronema*. However, *Uronema*, *Parauronema virginianum* and *Entodiscus borealis* always group together, which is concordant with previous studies (Lynn and Strüder-Kypke 2005, Gao *et al.* 2012a). *Parauronema* and *Uronema* have been suggested as junior synonym based on both the morphological and molecular data (Foissner 1971, Gao *et al.* 2012a). The plausible explanations for the grouping of the species *Entodiscus borealis* are that either this sequence is a contamination or the characters for the classification of scuticociliates are not reliable (Lynn and Strüder-Kypke 2005).

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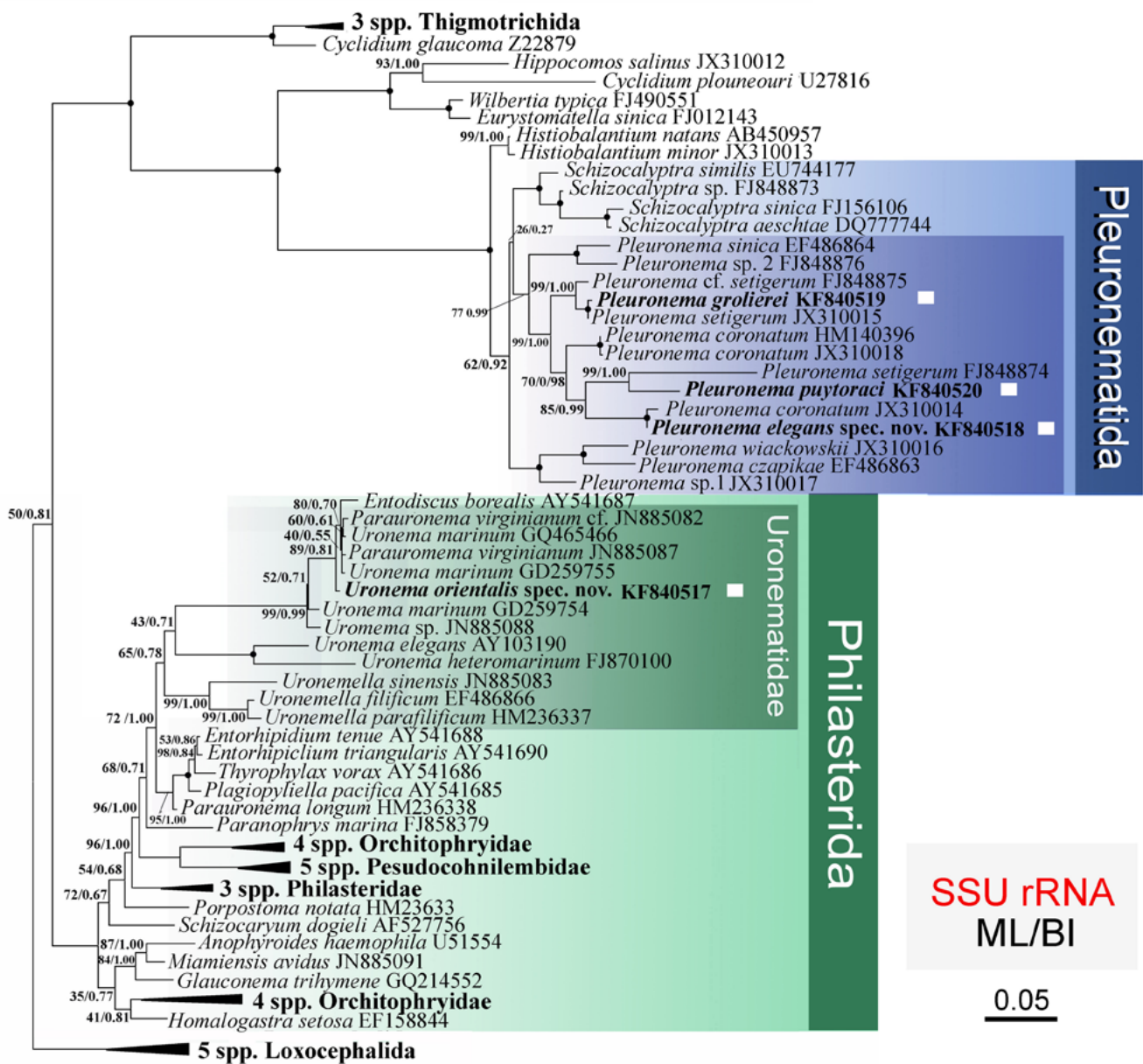


Fig. 5. Phylogenetic tree inferred from the small subunit ribosomal RNA (SSU rRNA) gene sequences, showing the positions of *Pleuronema elegans* spec. nov., *P. grolierei*, *P. puytoraci*, and *Uronema orientalis* spec. nov. (in bold). Numbers at nodes represent the bootstrap values of maximum likelihood (ML) out of 1,000 replicates and the posterior probability of Bayesian analysis (BI). The scale bar corresponds to five substitutions per 100 nucleotide positions.

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